PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 June 2001 (18.06.01)	
International application No. PCT/EP00/08270	**Applicant's or agent's file reference** 00/121
International filing date (day/month/year) 22 August 2000 (22.08.00)	**Priority date** (day/month/year) 22 August 1999 (22.08.99)
Applicant THUNNISSEN, Fredericus, Bernardus, Josephus, Maria et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 March 2001 (08.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Olivia TEFY

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
00/121		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08270	22/08/2000	22/08/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant DOT DIAGNOSTICS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/03/2001	26.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Wieser, M Telephone No. +49 89 2399 8434

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/08270

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 9
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 9

In view of the wording of claim 9 presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely claims 1-8.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70) 14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
00/121		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08270	22/08/2000	22/08/1999

International Patent Classification (IPC) or national classification and IPC
C12Q1/68

Applicant
DOT DIAGNOSTICS B.V. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/03/2001	26.11.2001

Name and mailing address of the international preliminary examining authority:
Europ an Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Wieser, M
Telephone No. +49 89 2399 8434

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08270

I. Basis of the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-25	as originally filed				

Claims, No.:

1-11	as received on	05/11/2001	with letter of	02/11/2001	

Drawings, sheets:

1/10-10/10	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 9.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 9.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-8,10,11

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-8,10,11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8,10,11
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Section III

Claims relating to inventions in respect of which no International Search Report has been established need not to be the subject of International Preliminary Examination (Rule 66(1)(e) PCT. Accordingly, claim 9, which is identified in the International Search Report as not having been searched, is not subject of this International Preliminary Examination Report.

Section V

The following documents mentioned in the International Search Report are considered as being the most relevant prior art:

(A) WO-A-96/41 005
(B) NUCLEIC ACIDS RESEARCH; vol. 22, no. 22, November 1994, pages 4840-4841, XP002013669
(C) WO-A-96/41 011
(D) WO-A-98/59 243

Claims 1-8,10, and 11 refer to a method, a device and a kit for detecting the presence of a nucleotide sequence within a dsDNA in a sample. The method is characterized in claim 1 by working steps (a) to (h), wherein steps (c) to (h) are performed by microarray technique on a solid glass support.

Documents A and B (from the Applicants of the preset application), refer to the method of claim 1 (i.e. the point-EXACCT method), with the difference that according to claim 1, the capturing step is carried out on a solid glass support coated with a first biotin layer to which, after drying, a second streptavidin layer is attached. In document A the exonuclease treated DNA oligonucleotide hybrids are captured on streptavidin-coated magnetic microspheres or on streptavidin-coated microspheres packed in columns. Other streptavidin-coated support matrices are said to be under investigation (see claims and example 2).

The problem to be solved by the present application was to improve the point-EXACCT method in order to perform large scale nucleic acid analysis, enabling

simultaneous analysis of thousands of DNA sequences (see page 3 of the description).

Chip- or microarray technology using Biotin-(Strept)avidin coated arrays is known in the art. Document C (see page 33) discloses a glass surface derivatized with an alkylamino linker which may be cross-linked to avidin to form an avidinated surface. Document D (see claims) refers to a method for attaching biotin covalently, i.e. by a photoactivation process, to a silicon dioxide support, and attaching streptavidin to immobilized biotin layer.

Thus, none of these documents would encourage a skilled person to amend the teaching in documents A and B in a way to arrive at the subject-matter of claims 1-8,10 and 11 in an obvious way. These claims meet the requirements of Article 33(3) PCT.

Section VIII

Claim 8 item (a), referring to a device, should contain a reference to claim 7 (Article 6 PCT).

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corrected version

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
00/121		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 08270	22/08/2000	22/08/1999
Applicant		
DOT DIAGNOSTICS B.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/08270

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention provides an improved method of detecting the presence of a nucleotide sequence within a double-stranded DNA in a sample comprising the following steps: a) coating a solid support with a first layer of biotinylated serum albumin, and a second layer of streptaviding having sufficient density to perform efficient microarray analysis; b) digesting the double-stranded DNA with an exonuclease to convert double-stranded to single stranded DNA, derived form a mixture of target cells and other cells, to a single-stranded DNA; c) capturing a first nucleic acid probe adapted by biotin to said coated solid support defined in step a.; d) hybridizing (i) the single-stranded DNA with the first nucleic acid probe, and (ii) a second nucleic acid probe labeled with a detectable moiety which can hydbridize with the single-stranded DNA adjacent the hybridized first nucleic probe; e) ligatin the hybridized first and second nucleic acid probes in case of perfect match only; f) denaturing the ligated first and second nucleic acid probes from the hybridized single-stranded DNA; g) removing non-covalently bound labeled probes and single stranded DNA; and h) detecting captured detectable moiety indicating he presence of the nucleotide sequence within the double-stranded DNA in the sample; wherein steps c.-h. are performed by microarray technique. Also provided is a device and a kit suitable for carrying out said detection method.

US

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
00/121		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 08270	22/08/2000	22/08/1999
Applicant		
DOT DIAGNOSTICS B.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/08270

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 9
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/08270

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9641005	A	19-12-1996	US	5744306 A	28-04-1998
			AU	6256896 A	30-12-1996
WO 9731256	A	28-08-1997	AU	2799797 A	10-09-1997
			CA	2244891 A	28-08-1997
			EP	0920440 A	09-06-1999
WO 9727317	A	31-07-1997	AU	2253397 A	20-08-1997
			EP	0880598 A	02-12-1998
WO 9411530	A	26-05-1994	US	5795714 A	18-08-1998
			EP	0668932 A	30-08-1995
			JP	8507199 T	06-08-1996
			US	6007987 A	28-12-1999
			US	5503980 A	02-04-1996
			US	5631134 A	20-05-1997
WO 9859243	A	30-12-1998	AU	8267998 A	04-01-1999
US 5242794	A	07-09-1993	US	4883750 A	28-11-1989
			US	5521065 A	28-05-1996
			US	5962223 A	05-10-1999
			DE	3586090 A	25-06-1992
			EP	0185494 A	25-06-1986
			JP	1919077 C	07-04-1995
			JP	6044880 B	15-06-1994
			JP	61191964 A	26-08-1986
WO 9641011	A	19-12-1996	US	5635400 A	03-06-1997
			AU	718357 B	13-04-2000
			AU	6102096 A	30-12-1996
			CA	2222581 A	19-12-1996
			CN	1193357 A	16-09-1998
			CZ	9703926 A	17-06-1998
			EP	0832287 A	01-04-1998
			HU	9900910 A	28-07-1999
			JP	11507528 T	06-07-1999
			NO	975744 A	05-02-1998
			PL	324000 A	27-04-1998
			AU	712929 B	18-11-1999
			AU	4277896 A	06-05-1996
			AU	7717596 A	30-04-1997
			CZ	9700866 A	17-09-1997
			EP	0793718 A	10-09-1997
			EP	0931165 A	28-07-1999
			FI	971473 A	04-06-1997
			JP	10507357 T	21-07-1998
			NO	971644 A	02-06-1997
			WO	9713877 A	17-04-1997
US 5710000	A	20-01-1998	US	6027894 A	22-02-2000

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 9

In view of the wording of claim 9 presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely claims 1-8.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

PATENT COOPERATION TREATY

From th
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Huygens, Arthur V.
OCTROOIBUREAU HUYGENS
P.O. Box 86
NL-3400 AB Ijsselstein
PAYS-BAS

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	26.11.2001
Applicant's or agent's file reference: 00/121	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08270	22/08/2000	22/08/1999

Applicant: DOT DIAGNOSTICS B.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Neumann, M Tel.+49 89 2399-7351

Form PCT/IPEA/416 (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inten nal Application No
PCT/EP 00/08270

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9641005 A	19-12-1996	US 5744306 A	28-04-1998
		AU 6256896 A	30-12-1996
WO 9731256 A	28-08-1997	AU 2799797 A	10-09-1997
		CA 2244891 A	28-08-1997
		EP 0920440 A	09-06-1999
WO 9727317 A	31-07-1997	AU 2253397 A	20-08-1997
		EP 0880598 A	02-12-1998
WO 9411530 A	26-05-1994	US 5795714 A	18-08-1998
		EP 0668932 A	30-08-1995
		JP 8507199 T	06-08-1996
		US 6007987 A	28-12-1999
		US 5503980 A	02-04-1996
		US 5631134 A	20-05-1997
WO 9859243 A	30-12-1998	AU 8267998 A	04-01-1999
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Section III

Claims relating to inventions in respect of which no International Search Report has been established need not to be the subject of International Preliminary Examination (Rule 66(1)(e) PCT. Accordingly, claim 9, which is identified in the International Search Report as not having been searched, is not subject of this International Preliminary Examination Report.

Section V

The following documents mentioned in the International Search Report are considered as being the most relevant prior art:

(A) WO-A-96/41 005
(B) NUCLEIC ACIDS RESEARCH; vol. 22, no. 22, November 1994, pages 4840-4841, XP002013669
(C) WO-A-96/41 011
(D) WO-A-98/59 243

Claims 1-8,10, and 11 refer to a method, a device and a kit for detecting the presence of a nucleotide sequence within a dsDNA in a sample. The method is characterized in claim 1 by working steps (a) to (h), wherein steps (c) to (h) are performed by microarray technique on a solid glass support.

Documents A and B (from the Applicants of the preset application), refer to the method of claim 1 (i.e. the point-EXACCT method), with the difference that according to claim 1, the capturing step is carried out on a solid glass support coated with a first biotin layer to which, after drying, a second streptavidin layer is attached. In document A the exonuclease treated DNA oligonucleotide hybrids are captured on streptavidin-coated magnetic microspheres or on streptavidin-coated microspheres packed in columns. Other streptavidin-coated support matrices are said to be under investigation (see claims and example 2).

The problem to be solved by the present application was to improve the point-EXACCT method in order to perform large scale nucleic acid analysis, enabling

simultaneous analysis of thousands of DNA sequences (see page 3 of the description).

Chip- or microarray technology using Biotin-(Strept)avidin coated arrays is known in the art. Document C (see page 33) discloses a glass surface derivatized with an alkylamino linker which may be cross-linked to avidin to form an avidinated surface. Document D (see claims) refers to a method for attaching biotin covalently, i.e. by a photoactivation process, to a silicon dioxide support, and attaching streptavidin to immobilized biotin layer.

Thus, none of these documents would encourage a skilled person to amend the teaching in documents A and B in a way to arrive at the subject-matter of claims 1-8,10 and 11 in an obvious way. These claims meet the requirements of Article 33(3) PCT.

Section VIII

Claim 8 item (a), referring to a device, should contain a reference to claim 7 (Article 6 PCT).

Revised Claims
(2.November 2001)

1. A method of detecting the presence of a nucleotide sequence within a double-stranded DNA in a sample comprising the following steps:

a. coating a solid glass support with a first layer of biotinylated serum albumin in an amount to create sufficient binding sites for the capture probes, drying said first layer and incubating said first dried layer with a second layer of streptavidin having sufficient density to perform efficient microarray analysis;

b. digesting the double-stranded DNA with an exonuclease to convert double-stranded to single stranded DNA, derived from a mixture of target cells and other cells, to a single-stranded DNA;

c. capturing a first nucleic acid probe adapted by biotin to said coated solid support defined in step a.;

d. hybridizing (i) the single-stranded DNA with the first nucleic acid probe, and (ii) a second nucleic acid probe labeled with a detectable moiety which can hybridize with the single-stranded DNA adjacent the hybridized first nucleic probe;

e. ligating the hybridized first and second nucleic acid probes in case of perfect match only;

f. denaturing the ligated first and second nucleic acid probes from the hybridized single-stranded DNA;

g. removing non-covalently bound labeled probes and single stranded DNA; and

h. detecting captured detectable moiety indicating the presence of the nucleotide sequence within the double-stranded DNA in the sample;

characterized in that steps c.-h. are performed by microarray technique.

2. The method of claim 1, wherein step d (ii) is adapted with the use of a mixture of partly randomized probes to allow detection of mutations without knowing the site and type of mutation beforehand.

3. The method according to claim 1 or claim 2, wherein said solid glass support is made of Starfrost glass.

4. Th method of any on of claims 1 to 3, wherein first nucleic acid probes are printed on said solid glass support or are built on said solid glass support by light-directed oligonucleotide synthesis.

5. The method of any one of claims 1 to 4, wherein the detectable moiety on the second nucleic acid probe is digoxigenin, and the detecting step is performed by binding the digoxigenin with anti-digoxigenin antibody fragments.

6. A device suitable for carrying out the detection method as claimed in any one of the preceding claims, which comprises a solid glass support having a coating which is obtainable by the method comprising coating said solid glass support with a first layer of biotinylated serum albumin in an amount to create sufficient binding sites for the capture probes, drying said first layer, and incubating said first dried layer with a second layer of streptavidin having sufficient density to perform efficient microarray analysis.

7. The device according to claim 6, wherein the said solid glass support is made of Starfrost glass.

8. A kit comprising:

a. a device suitable for carrying out the detection method according to the present invention as claimed in any one of claims 1 to 7;

b. optionally an exonuclease;

c. a first nucleic acid probe which binds to target DNA and which is adapted with a capture moiety;

d. a second nucleic acid probe which binds to target DNA adjacent the first probe and which is labeled with a detectable moiety; and

e. optionally a ligase.

9. A method for organizing microarray analysis on a solid glass support for rapid visual detection of abnormalities which comprises arranging a duplicate set of probes where the first series of arrays are for the wild-type mutation order and the second series of arrays are for the classical sequencing order.

10. The method of claim 1, wherein prior to th drying in step a parafilm, preferably covered by a weight, or a surfactant is added to enhance the distribution of said first layer.

11. The device of claim 6, wherein the solid glass support is obtainable by the method in which prior to the drying in step a parafilm, preferably covered by a weight, or a surfactant is added to enhance the distribution of said first layer.

Claims

1. A method of detecting the presence of a nucleotide sequence within a double-stranded DNA in a sample comprising the following steps:

a. coating a solid support with a first layer of biotinylated serum albumin, and a second layer of streptavidin having sufficient density to perform efficient microarray analysis;

b. digesting the double-stranded DNA with an exonuclease to convert double-stranded to single stranded DNA, derived from a mixture of target cells and other cells, to a single-stranded DNA;

c. capturing a first nucleic acid probe adapted by biotin to said coated solid support defined in step a.;

d. hybridizing (i) the single-stranded DNA with the first nucleic acid probe, and (ii) a second nucleic acid probe labeled with a detectable moiety which can hybridize with the single-stranded DNA adjacent the hybridized first nucleic probe;

e. ligating the hybridized first and second nucleic acid probes in case of perfect match only;

f. denaturing the ligated first and second nucleic acid probes from the hybridized single-stranded DNA;

g. removing non-covalently bound labeled probes and single stranded DNA; and

h. detecting captured detectable moiety indicating the presence of the nucleotide sequence within the double-stranded DNA in the sample;

characterized in that steps c.-h. are performed by microarray technique.

2. The method of claim 1, wherein step d (ii) is adapted with the use of a mixture of partly randomized probes to allow detection of mutations without knowing the site and type of mutation beforehand.

3. The method according to claim 1 or claim 2, wherein said solid support is made of glass, preferably Starfrost glass.

4. The method of any one of claims 1 to 3, wherein first nucleic acid probes are printed on said solid support or are built on said solid supp rt by light-directed oligonucleotide synthesis.

5. The method of any one of claims 1 to 4, wh rein the detectable moi ty on the second nucl ic acid probe is digoxigenin, and the detecting step is performed by binding the digoxigenin with anti-digoxigenin antibody fragments.

6. A device suitable for carrying out the detection method as claimed in any one of the preceding claims, which comprises a solid support having a coating comprising a first layer of biotinylated serum albumin, and a second layer of streptavidin having sufficient density to perform efficient microarray analysis.

7. The device according to claim 8, wherein the said solid support is made of glass, preferably Starfrost glass.

8. A kit comprising:

a. a device suitable for carrying out the detection method according to the present invention as claimed in any one of claims 1 to 7;

b. optionally an exonuclease;

c. a first nucleic acid probe which binds to target DNA and which is adapted with a capture moiety;

d. a second nucleic acid probe which binds to target DNA adjacent the first probe and which is labeled with a detectable moiety; and

e. optionally a ligase.

9. A method for organizing microarray analysis on a solid support for rapid visual detection of abnormalities which comprises arranging a duplicate set of probes where the first series of arrays are for the wild-type mutation order and the second series of arrays are for the classical sequencing order.

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-8,10,11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8,10,11
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 9.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-8,10,11

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08270

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-25	as originally filed				
Claims, No.:					
1-11	as received on	05/11/2001	with letter of	02/11/2001	
Drawings, sheets:					
1/10-10/10	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08270

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 41005 A (MURTAGH JAMES J ;THUNNISSEN FREDERIK B J M (NL)) 19 December 1996 (1996-12-19) cited in the application the whole document	1-8
X	SOMERS V A M C ET AL: "A RAPID, RELIABLE METHOD FOR DETECTION OF KNOWN POINT MUTATIONS: POINT-EXACCT" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 22, no. 22, 11 November 1994 (1994-11-11), pages 4840-4841, XP002013669 ISSN: 0305-1048 cited in the application the whole document	1-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January 2001	31 01 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08270

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 31256 A (BLOK HERMAN ;BARANY GEORGE (US); KEMPE MARIA (US); ZIRVI MONIB (US) 28 August 1997 (1997-08-28) cited in the application the whole document	1-8
Y	WO 97 27317 A (CHEE MARK ;LAI CHAOQIANG (US); LEE DANNY (US); AFFYMETRIX INC (US)) 31 July 1997 (1997-07-31) cited in the application See "VIII. Ligation enhanced signal detection", page 70-page 83 the whole document	1-8
Y	WO 94 11530 A (UNIV BOSTON) 26 May 1994 (1994-05-26) the whole document	1-8
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Y	US 5 242 794 A (HUNKAPILLER MICHAEL W ET AL) 7 September 1993 (1993-09-07) cited in the application the whole document	1-8
Y	WO 96 41011 A (SPECTRAGEN INC) 19 December 1996 (1996-12-19) See Fig.3, claims 46-51 the whole document	1-8
A	US 5 710 000 A (GINGERAS THOMAS R ET AL) 20 January 1998 (1998-01-20) the whole document	

2

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(48) Date of publication of this corrected version:
3 May 2001

(15) Information about Correction:
see PCT Gazette No. 18/2001 of 3 May 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IMPROVED METHOD FOR NUCLEOTIDE DETECTION AND DEVICES USED THEREIN

(57) Abstract: The present invention provides an improved method of detecting the presence of a nucleotide sequence within a double-stranded DNA in a sample comprising the following steps: a) coating a solid support with a first layer of biotinylated serum albumin, and a second layer of streptaviding having sufficient density to perform efficient microarray analysis; b) digesting the double-stranded DNA with an exonuclease to convert double-stranded to single stranded DNA, derived from a mixture of target cells and other cells, to a single-stranded DNA; c) capturing a first nucleic acid probe adapted by biotin to said coated solid support defined in step a; d) hybridizing (i) the single-stranded DNA with the first nucleic acid probe, and (ii) a second nucleic acid probe labeled with a detectable moiety which can hybridize with the single-stranded DNA adjacent the hybridized first nucleic probe; e) ligating the hybridized first and second nucleic acid probes in case of perfect match only; f) denaturing the ligated first and second nucleic acid probes from the hybridized single-stranded DNA; g) removing non-covalently bound labeled probes and single stranded DNA; and h) detecting captured detectable moiety indicating the presence of the nucleotide sequence within the double-stranded DNA in the sample; wherein steps c.-h. are performed by microarray technique. Also provided is a device and a kit suitable for carrying out said detection method.

WO 01/18241 A1